Differential expression of growth-arrest specific 1, GAS1, in the brain metastases of patients with breast cancer.

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Metastasis to the brain is a complication of breast cancer^{1,2} with limited treatment options³. We mined published microarray data^{4,5} to discover genes associated with brain metastases in patients with metastatic breast cancer. We identified significant differential expression of GAS1 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast. GAS1 may be relevant to the underlying biology by which tumor cells of the breast spread to the brain.

Keywords: GAS1, breast cancer, brain metastases, central nervous system metastases, systems biology of breast cancer, targeted therapeutics in breast cancer.

One study documented a 34% incidence of metastases to the central nervous system in breast cancer patients treated with trastuzumab⁶. An unbiased and systems-level understanding of the transcriptional makeup of brain metastatic tissues can support identification of therapeutic targets. We performed global transcriptional profiling of brain metastases in patients with brain metastatic breast cancer⁶⁻⁸. We discovered significant differential expression of the growth-arrest specific gene GAS1 in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE26338⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=16$ primary tumors from patients with breast cancer and $n=16$ brain metastases from patients with brain metastatic breast cancer. GSE26338 was generated using Human 1A Microarray G4110A technology with $n=11$ primary breast tumors and $n=3$ brain metastases from patients with breast cancer, analysis performed using platform GPL885. The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether GAS1 gene expression was significantly between primary breast tumors and brain metastases in humans with breast cancer using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455).

Results

We sought to discover genes associated with metastasis to the brain in patients with breast cancer by mining published microarray datasets^{4,5}, comparing global gene expression profiles of primary tumors of the breast to brain metastatic tissues from patients with breast cancer.

GAS1 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the gene encoding growth-arrest specific 1, GAS1, as among the genes whose expression was most quantitatively different in brain metastases when compared to primary tumors of the breast in patients with breast cancer⁴. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to primary tumors of the breast, GAS1 ranked 4 out of 22277 total transcripts (Table 1). Differential expression of GAS1 in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=2.26E-07$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of GAS1 in brain metastatic breast cancer. We again found that GAS1 was among the genes whose expression was most significantly different when comparing primary tumors of the breast to brain metastases from patients with metastatic breast cancer. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to primary tumors of the breast, GAS1 ranked 15 out of 22575 total transcripts (Table 1). Differential expression of GAS1 in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=1.18E-05$).

GAS1 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer as compared to primary tumors of the breast.

We obtained exact mRNA expression levels for GAS1 in primary tumors of the breast and in brain metastases to evaluate magnitude and direction of change in GAS1 gene expression in brain metastatic tissues. In both datasets queried, GAS1 was expressed at lower levels in brain metastases from patients with breast cancer as compared to primary tumors of the breast (Figure 1 and Figure 2). Decreased expression of GAS1 in brain metastases as compared to primary tumors of the breast was statistically significant (Figure 1 and Figure 2; $p < 0.0001$ and $p < 0.0001$, respectively). We calculated a mean fold change of 0.638 ± 0.204 in GAS1 expression in brain metastases when compared to primary tumors of the breast (Table 1).

Thus, through comparative transcriptome analysis of tumor and metastatic tumor tissues of patients with breast cancer using published microarray data^{4,5}, we found that GAS1 was among the genes most differentially expressed in brain metastases, with significantly decreased expression in brain metastatic tissues as compared to primary tumors of the breast.

Discussion

To facilitate discovery of novel therapeutic targets in brain metastatic breast cancer, and to more thoroughly understand the biology of metastasis to the brain in breast cancer, we mined published microarray data^{4,5} to identify genes most differentially expressed in the brain metastases of patients with metastatic breast cancer.

GAS1 is described as an integral membrane protein⁶ with tumor suppressive and growth regulatory properties⁶⁻⁹; GAS1 expression blocks cell cycle transition from G0 to S phase induced by serum⁶. Expression of GAS1 can be suppressed by Myc¹⁰ and its function intersects with that of the p53 tumor suppressor pathway¹¹. We report here that GAS1 is present in significantly lower quantities in brain metastases in patients with metastatic breast cancer and that GAS1 is among the genes whose expression changes most significantly transcriptome-wide in brain metastatic tissues from patients with brain metastatic breast cancer. Compromised growth control functions of GAS1 due to partial loss of expression can be explored to understand mechanisms by which metastatic tumor cells travel to the brain, colonize the brain, and survive in foreign tissues.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
4	204457_s_at	2.26E-07	-6.426424	6.6367	0.638 ± 0.204	GAS1	growth arrest specific 1

Table 1: Differential expression of GAS1 in brain metastases in patients with metastatic breast cancer.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, fold change (FC) of GAS1 expression between the brain metastases of patients with breast cancer and primary tumors of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
15	15049	1.18E-05	-6.46	3.49242	GAS1	growth arrest-specific 1

Table 2: Differential expression of GAS1 in brain metastases in patients with metastatic breast cancer.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Figure 1: GAS1 is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in patients with brain metastatic breast cancer.

The mRNA expression level of GAS1 in primary tumors of the breast (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean GAS1 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and primary tumors of the breast, a *p*-value, listed above.

Figure 2: GAS1 is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in patients with brain metastatic breast cancer.

The mRNA expression level of GAS1 in primary tumors of the breast (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean GAS1 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and primary tumors of the breast, a p -value, listed above.